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Slippage of DNA polymerase I during synthesis of ds-cDNA

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Synthesis of ds-cDNA copies of mRNA usually involves oligo-dT primed first strand synthesis followed by the RNase H-DNA polymerase I mediated second strand synthesis (1, 2). The mechanism whereby under these conditions the RNA strand is replaced with DNA probably involves nicking - followed by displacement of the RNA strand. Few intermediate length second strand products are generated starting from homogeneous cDNA.RNA hybrid and no ligase is required to synthesize full length ds-cDNA. This means that within a short time, only a small RNA segment complementary to the 5' region of the cDNA is left over by the RNase H attack to function as a primer for the DNA polymerase I. Using unlabelled globin cDNA.RNA hybrid for synthesis of ds-cDNA some product is also generated using the 5' cDNA-hairpin loop as a primer. Such molecules migrate in an alkaline agarose gel with a size twice that of the original cDNA (Figure). In addition to the full length second strand globin DNA, we also observed products between 100 and 300 nucleotides in length in the reactions containing [α-^{32}P]dATP (or [α-^{32}P] dTTP, not shown) as a labelled triphosphate (lane 2, Figure). These products are absent in the reactions containing [α-^{32}P] dCTP (or [α-^{32}P] dGTP, not shown) as the labelled triphosphate (lane 3 and 4, Figure). This means that the DNA polymerase can initiate a slippage poly-dA.poly-dT synthesis starting on the oligo-dT, or on the poly-dT generated by slippage of the reverse transcriptase during the first strand synthesis (3, 4).

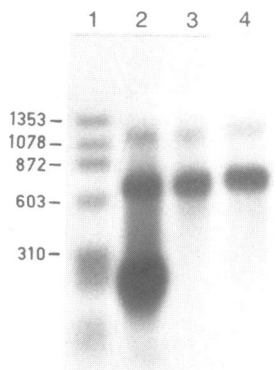

Figure : Analysis of second strand globin cDNA products synthesized according to Gubler and Hoffman (2) in the presence of different [α-^{32}P] dNTPs. Samples were analyzed on a 2% alkaline agarose gel. Markers were 5' ^{32}P-labeled HaeIII fragments of ϕX174 DNA. Lane 1 : markers; Lane 2 : 2nd strand synthesized in the presence of [α-^{32}P] dATP; Lane 3 : [α-^{32}P] dCTP; Lane 4 : [α-^{32}P] dCTP, DNA ligase and β-NAD.

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